

Localization of Ischemic Brain Region After Mouse Embryonic Stem Cell Transplantation

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ABSTRACT

We induced middle cerebral artery occlusion (MCAO) in rats using silicone-coated vascular embolus. We transplanted mouse embryonic stem cells after MCAO. Rats were tested behaviorally using motor and sensory function with neurological assessment. Functional effectiveness of the mES transplanted was gradually improved the function of sensory neuron and motor neuron. This study demonstrated that the transplanted cells have synaptic connection in the recipient brain. We suggested that stem cell transplantation can have a positive effect on behavioral recovery and reduction of infarct size in focal ischemic rats. Therefore, we concluded that mES cells may have useful tool for treatment in neurological diseases.

Key words: mESc(mouse embryonic stem cell), Sensory neuron, Motor neuron, Infarct

INTRODUCTION

Cerebral ischemia model induces a severe damage of neuron, and it causes behavioral dysfunction in rats [1]. The criteria of functional recovery in stroke patients being the main issue, this gradual enlargement of infarction can be limited by a variety of interventions that do not interfere with cerebral blood flow. Therefore, we performed an analysis to investigate the evolution of infarct after MCAO. We induced this analysis in rats, a model system that has been less well characterized. In order to analyze the changes following transient MCAO, several different histochemical methodologies can be utilized. TTC staining is one of the most common histochemical stains used to assess cerebral injury. In ischemic tissue, lack of TTC staining is considered infarcted with white color and defined as core and viable tissue is stained red [7,9-10]. We examined the effects of the transplantation of mouse ES cells on behavioral function induced by focal ischemia in rats. Stem cell transplantation has established as a potential effective therapy for CNS disorders such as ischemic stroke and spinal cord injury. Embryonic stem (ES) cells are capable of proliferating and differentiation into neural progenitor cells with the use of induction protocols leading to the development of functionally mature neurons and glial cells [2]. Self-renewing, totipotent embryonic stem (ES) cells may use a virtually unlimited donor source for tissue transplantation [3]. We have focused on determining the

appropriate culture condition to induce neural cell differentiation of ES cells with good cell viability. A cell based-therapy may have the advantage of exerting multiple therapeutic effects at various sites and times within the lesion as the cells respond to a particular pathological microenvironment. Although a single injection of mESCs several hours after ischemia onset can reduce infarction size and improve functional outcome in rodent cerebral ischemia models [4-5]. Although endogenous neurogenesis and migration of precursor cells may help to replace some lost neurons in brain structures such as striatum [6], transplantation of embryonic stem cells remains to be the most liable way to repair the massive damage in the cerebral cortex after ischemic stroke.

MATERIALS AND METHODS

Animal model

Rat models with MCAO were randomly assigned to one of two groups (n=20 for each group): Rats were divided into mESC transplantation (group A, n=20) and PBS-only injection (group B, n=20).

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Cerebral ischemic model

This study was approved by the animal care and use committee of Namseoul University, and all procedures were carried out in accordance with institutional guidelines. We induced permanent MCAO by using a previously described method of intraluminal vascular occlusion. Adult female Sprague-Dawley rats (n=36) weighing 250–300 g were anesthetized with an intraperitoneal (i.p.) injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). We induced transient left middle cerebral artery occlusion (MCAO) for 120 min as previously described [11]. After the occlusion for 30 minutes, the filament was withdrawn.

Transplantation procedure and staining

After anesthesia with an intraperitoneal injection of ketamine hydrochloride (90 mg/kg), the rats were given 10.0- μ l deposits of suspended cells (1×10^5 cells per μ l, or 1.0 μ l of buffer vehicle only) along the anterior-posterior axis into the target brain at these coordinates: from the bregma, 1.5 mm laterally and to 3 mm depth. Rats were euthanized at 28 days of reperfusion. The brains were chilled at -80°C for 4min to slightly harden the tissue. Five, 2-mm coronal sections were made from the olfactory bulb to the cerebellum and then stained with 1.5% TTC (Sigma, St. Louise, MO). The stained brain sections were captured with a digital camera. GFP-gene transfection to mES cells were detected in vivo using a fluorescence microscope.

Statistical analysis

Quantitative data were expressed as mean \pm SEM. Two-way ANOVA and Student's t test with the Bonferroni correction for multiple pair-wise comparisons were used for statistical analysis.

p values <0.05 were considered significant.

RESULTS

fMRI characteristics of infarction region

White color of infarction region was shown mainly in corpus callosum & striatum. Figure 1 shows severe inflammation of infarction site with cytotoxic edema by diffusion coefficient in day 5 after MCAO. fMR imaging technique can be recognized specific infarction region in vitro.

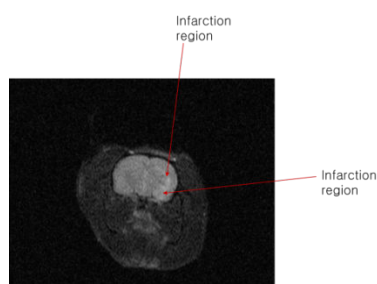


Figure 1: fMR imaging of infarction region showed in more white site in vitro.

Localization of ischemic lesion

Normal brain (gray matter) tissue typically stains with TTC, but infarcted lesions show no or reduced staining. TTC staining obtained 4 weeks after MCAO without cell transplantation is shown in Figure B. Note the reduced staining on the lesion side primarily in the corpus striatum. There was a progressive reduction in infarction size with mESC treatment. Intracerebral delivery of mESCs resulted in very substantial reduction in lesion volume as estimated from TTC staining. Cell treatment reduces MCAO-induced brain infarction. Representative TTC stained brain sections are shown where rats were injected with PBS (A; n=18) or mESC (B; n=18) before MCAO. Animals were killed 24 h later and the brains were sliced into 2 mm sections and stained with 2,3,5-triphenyltetrazolium chloride (TTC). Infarct volumes in brains from PBS and mESC treated animals are shown in the graph (Figure 2. A,B).

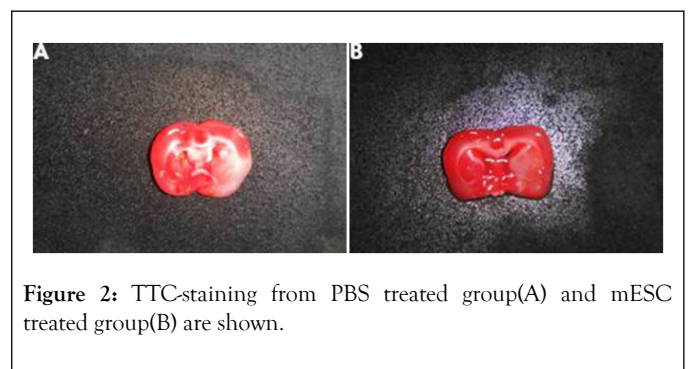


Figure 2: TTC-staining from PBS treated group(A) and mESC treated group(B) are shown.

DISCUSSION

It has been reported that transplantation of mouse ES cells into rat brain following experimental stroke reduced infarct volume and improved behavioral outcome [10]. In the present study, it is reported that transplantation also stimulated neurogenesis in the SVZ ipsilateral to stroke [13]. Because the magnitude of the inflammatory response and its harmful effects as well as the types of released cytokines change with time after ischemia [14-15], the timing of the transplant could significantly influence graft survival, and longer survival could be predicted if cells are transplanted once inflammation has subsided. The present study shows that transplantation of mESC not only increased early survival of transplanted cells but also accelerated behavioral recovery following stroke. This is consistent with previous report that grafted embryonic stem cells develop into functional neurons and could integrate into host cortical circuitry [16-18]. The functional recovery 7 days after transplantation suggests the therapeutic advantage of accelerated repair processes and functional restoration. The motor functional benefit, however, was more persistent in mESC transplantation, showing much better performance than control animals at 28 days after MCAO. More long-term investigation may be needed to verify the persistence of the morphological and functional benefits of the transplantation strategy [2]. Our finding showed that the infarct size was significantly reduced in both cell-transplanted groups and the post-ischemic exercise group, compared with the sham-operated group. Although the basis for the propagation of injury is unclear, it is well known that an ischemic brain infarct progresses over time [19].

CONCLUSION

Cell transplantation may induce certain functional recovery of the brain tissue by endogenous cell mediated effect. Our study suggested that intracerebrally mES cells survived, migrated into the infarct area from inoculation site and neuroglially differentiated in the ischemic brain of adult rats.

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